ATCC

Product Sheet

# *Crithidia confusa* (ATCC® PRA-346™)

**Please read this FIRST**

Storage Temp.

***Frozen Cultures:***
**-70°C for 1 week; liquid $N_2$ vapor for long term storage**

***Freeze-dried Cultures:***
**2-8°C**

***Live Cultures:***
**See Protocols section for handling information**

Biosafety Level
**1**

## Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

## Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Crithidia confusa* (ATCC® PRA-346™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

*Or contact your local distributor*

## Description

**Strain Designation:** 320AR
**Depositor:** D Maslov
**Isolation:** Intestine of *Largus* cf. *cinctus* (Heteroptera), Alajuela Province, Costa Rica, 2009

## Propagation

**Growth Conditions**
**Temperature:** 20°C to 25°C

**Medium**
ATCC® Medium 44: Brain Heart Infusion Agar/Broth

**Instructions for Complete Medium**
**Media:** ATCC medium 44 supplemented with 10 µg/mL hemin

## Protocols

**Storage and Culture Initiation**
Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampules may be stored at or below -70°C for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally -20°C).** Storage of frozen material at this temperature will result in the death of the culture.

1. To thaw a frozen ampule, place in a 35°C water bath, until thawed (2-3 min). Immerse the ampule just sufficiently to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer contents to a T-25 tissue culture flask containing 10.0 mL ATCC medium 44 supplemented with 10 µg/mL hemin. Incubate at 20-25°C with the cap screwed on tightly.

**Culture Maintenance**

1. Agitate a culture at or near peak density and aseptically transfer 0.1-0.2 mL to a fresh flask of ATCC medium 44 supplemented with 10 µg/mL hemin.
2. Incubate at 20-25°C with the cap screwed on tightly.
3. Transfer the culture every 7-10 days as described in steps 1-2. The transfer interval will depend on the quantity of the inoculum and the quality of the medium. This should be empirically determined by examining the culture on a daily basis until the growth cycle has stabilized.

## Cryopreservation

**Harvest and Preservation**

1. Harvest cells from a culture which is at or near peak density by centrifugation at ~800 x g for 5 min.
2. Adjust concentration of cells to 2 x $10^7$/mL in fresh medium. If the concentration is too low, centrifuge at ~800 x g for 5 minutes and resuspend the cell pellet with a volume of supernatant to yield the desired concentration.
3. While cells are centrifuging, prepare a 10% (v/v) solution of sterile DMSO in fresh medium (broth). The DMSO solution when first prepared will warm up due to chemical heat. The solution should be allowed to return to room temperature prior to use.
4. Mix the cell preparation and the DMSO solution in equal portions. The final concentration will be $10^7$ cells/mL and 5% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution before the freezing process is begun should be no less than 15 min and no more than 30 min.
5. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
6. Place the ampules in a Nalgene 1°C freezing apparatus. Place the apparatus at -80°C for 1.5 to 2 hours and then plunge ampules into liquid nitrogen. (The cooling rate in this apparatus is approximately -1°C/min.) If freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through heat of fusion. At -40°C, plunge ampules into liquid nitrogen.
7. Store in either the vapor or liquid phase of a nitrogen refrigerator.
8. To thaw a frozen ampule, place it in a 35°C water bath such that the lip of the ampule remains above the water line. Thawing time is approximately 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after it is thawed.

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9. Remove the vial from the water bath immediately after thawing. Aseptically transfer the contents of the ampule into a T-25 tissue culture flask containing 10.0 mL ATCC medium 44 supplemented with 10 µg/mL hemin.
10. Incubate the tube at 20-25°C with the cap screwed on tightly.
11. Maintain as described above.
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## References

References and other information relating to this product are available online at www.atcc.org.

## Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

## ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

## Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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